REMARKS

Detailed Action

Claims 1-12, 20-23, and 28-33 are pending.

Sequence Rules

The Examiner states the application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the following reasons:

(A) The sequence identifier used on page 7 does not match up with the sequence listing.

(B) The sequences recited in Fig. 7 must be identified with proper sequence identifiers.

(C) The nucleic acid sequences recited in Fig. 5 must be identified with proper sequence identifiers.

Pursuant to 37 CFR 1.821 (b), which states in part: "It should be noted, though, that when a sequence is presented in a drawing, regardless of the format or the manner of presentation of that sequence in the drawing, the sequence must still be included in the Sequence Listing and the sequence identifier (SEQ ID NO: X) must be used, either in the drawing or in the Brief Description of the Drawings." Thus, for Fig.7, Applicants have inserted the sequence identifier in the "Brief Description of the Drawing," thus alleviating this rejection.

The amendments to the sequence identifiers in the specification will enable the sequence identifiers to match up with the Sequence Listing.

More over pursuant to 37 CFR 1.821 (b), as recited above, Applicants have shown the sequence identifier in the "Brief Description of the Drawings," (See page 5, line 6) therefore, the sequence recited in Fig. 5 does not have to be identified with a proper sequence identifier.

Applicants are herein submitting a new CRF and paper copy of the Sequence Listing.

Please enter the newly submitted sequence listing into the specification.

Claim Rejections-35 USC § 112

Claims 1-12, 20-23, and 28-33 were rejected under 35 USC 112, first paragraph, because the specification, while being enabling for methods of identifying a pig which posses a genotype indicative of the pig having less back fat than pigs with a different genotype, indicative of the pig having a lower daily gain than pigs with a different genotype, or of pigs having a lower feed intake than a pig with a different genotype, wherein said method comprising screening DNA of the pig for a G to an A point mutation at position 678 of SEQ ID NO: 1 and wherein the absence of the mutation is indicative of a pig having the recited traits, does not reasonably provide enablement for methods which screen other animals or methods which utilize other polymorphisms. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

Applicants respectively traverse. Applicants have shown that polymorphism in the MC4R gene has been located and is associated with the metabolic traits of fat content, growth rate, and feed consumption in animals. Additionally, Applicants have shown that this particular polymorphism is within a highly conserved region among melanocortin receptors (MCR). The sequence conservation is such that it is recognizable for those skilled in the art to screen pigs and other animals for this polymorphism. The specification goes into great detail about how to identify the existence of a particular polymorphism in an animal. Once knowledge of the existing polymorphism is known, it would take no more than routine screening to identify the presence of the polymorphism in another species.

Moreover, the specification discloses a multiple alignment of the predicted amino acid sequences of the pig MC4R with the MC4R protein from other species. This alignment shows that the aspartic acid found a position 298 of the seventh transmembrane domain is very highly conserved in the MC4R protein among species.

Also, Applicants have shown that the MC4R gene consists of approximately a 1kb coding sequence contained within a single exon with the pig MC4R gene fragment being nearly the entire gene. More specifically, Applicants have shown that the marker for this metabolic trait observed in the specification is allele 1 and allele 2. Because of the evolutionary link between pigs and other species, it can be predicted that variation in this gene is also likely to be associated

with the same metabolic traits in these other species. This polymorphism can be identified in the MC4R gene of these other species using the same approach set out in the specification with the resulting single nucleotide polymorphism used for association analysis. Applicant's have disclosed the methods and techniques for screening for the existence of this polymorphism and it would require no more that routine experimentation to use the techniques disclosed in the application to ascertain if, in fact the identical polymorphism exist in other species, animals or populations. From the entire sequence of the MC4R gene applicant has discovered through diligent testing, the existence of a polymorphism that is associated with traits of economic interest. Once known, it is routine given the teachings in the specification to assay for this same polymorphism in different species.

The Examiner further states, the level of unpredictability and the level of experimentation required to expand the instantly disclosed methods to include animals of other species are also quite high. There is no teaching in the specification that the disclosed polymorphism even exists in animals of other species.

Applicants traverse. Applicants have shown a polymorphism in the MC4R gene has been located and is associated with the metabolic traits of fat content, growth rate, and feed consumption in animals. Additionally, Applicants have shown that this particular polymorphism is within a highly conserved region among melanocortin receptors. Moreover, the specification discloses a multiple alignment of the predicted amino acid sequences of the pig MC4R with the MC4R protein from other species. This alignment shows that aspartic acid found at position 298 of the seventh transmembrane domain is very highly conserved in the MC4R protein among species. Also, Applicants have shown that the MC4R gene consists of approximately 1 kb coding sequence contained with any single exon with the pig MC4R gene being nearly the entire gene. More specifically, Applicants have shown that the marker for these metabolic traits observed in the specification are alleles 1 and 2. Because of the evolutionary link between pigs and other species, it can be predicted that variation in this gene is also likely to be associated with the same metabolic traits in other species. This polymorphism can be identified in the MC4R gene of these other species using the same approach set out in the specification with the resulting single nucleotide polymorphism used for association analysis. Applicants submit that there would be no undue experimentation to screen for a potential polymorphism for a nucleotide

sequence in the MC4R gene and identify first whether or not it is polymorphic and then assay for whether there is a significant association with a trait of interest association with this polymorphism. Applicants have identified such a polymorphism and based upon this work any one of skill in the art based upon this information described by application's, easily screen for the identical polymorphism in other species using such programs as BLAST or Clustal. The MC4R gene has been shown to be highly polymorphic in other species such as mouse as disclosed in the specification. Applicants respectfully request Examiner to withdraw this rejection.

The Examiner further states that in order to provide such evidence, the skilled artisan would be required to undertake extensive studies of the metabolic traits of hundreds upon hundreds of different animals of each of many different species of animals. Such experimentation would be inventive in itself. Applicants agree with the Examiner that such random screening would be inventive in and of itself; in this case however that is not what is required. Applicants have disclosed the trait and the polymorphism. Applicants respectfully request Examiner to withdraw this rejection.

Claims 1-12, 20-23, 29, and 30 were rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regards as the invention.

Claim 1 was rejected as being indefinite because the purpose of the method as set forth in the preamble is unclear.

Claim 1 has been amended to identity to specify that animals possessing genotypes indicative of particular traits are identified.

Claims 2, 4, 5, and 6 were further indefinite over the recitation "at base 678 in a PCR sequence of the MC4R gene" because this language does not clearly identify where within the nucleotide sequence of the MC4R gene the polymorphism is being identified. The position of the nucleotide within a PCR product is entirely dependent upon the primers used to amplify the product. These claims do not indicate which primers are to be used and therefore, the designation "at base 678 in a PCR sequence of the MC4R gene" is arbitrary and fails to clearly identify the scope of the claim.

Applicants have amended claim 2 to recite the usage of primers. Moreover, Applicants have added claims, depending from claim 2, which recite specific primers used to clearly identify the position of the polymorphism in the PCR product. Claims 4, 5, and 6 because of there dependency on claim 2 includes all its limitations and therefore, recite what primers are used to identify where in the PCR product the polymorphism is located.

Claim 4 was rejected as being indefinite. The Examiner states it is not clear what portion of the claim "associated with variation in fat content" modifies.

Applicants have amended claim 4 by deleting any reference to "variation", thus alleviating this rejection.

Claim 12 was rejected as being indefinite. The Examiner states it is not clear if it is a product claim or a method claim since it depends from method claim 10, but begins with "the amplified sequence".

Applicants have canceled claim 12, thus alleviating this rejection.

Claim 20-23 were rejected as being indefinite for failing to recite a final process step which agreed back with the preamble.

Applicants have amended claims 20-23 to recite a final process step which agrees back with the preamble. Claims 22 and 23 by virtue of their dependency should be in allowable as they claim all the limitations of amended independent claim 20. Support for the amendment is found on page 8, lines 18-20 of the specification.

Claim 22 was rejected as being indefinite over the recitation of "at base 678 of the amplified product" because claim 21 recites two amplified products and it is not clear which amplified product the claim intends.

Applicants have amended claim 22 by removing the recitation of "at base 678 of the amplified product", thus alleviating this rejection.

Claim 22 is also indefinite over the recitation "when a restriction enzyme which cuts at the same recognition site as Taq I is used" because it is not clear when the restriction enzyme is used or what it is used to digest.

Applicants have amended claim 22 by deleting this recitation and making further amendments.

Claim 29 and 30 were rejected as being indefinite over the recitation "alternative DNA marker" because it is not clear what this marker is an alternative to.

Applicants have amended claim 29 and 30 to more clearly state what the "alternative DNA marker" is an alternative to a DNA marker that has already been known to be associated with the MC4R gene which has previously been shown to be associated with a particular trait, and by establishing linkage between specific alleles of an alternative DNA marker and alleles of DNA markers known to be associated with the MC4R gene, one skilled in the art would be able to indirectly select for the polymorphism with alternative DNA markers. Support for amendment is found on page 10, lines 31-36 to page 11, lines 1-2.

Claim Rejections-35 USC § 102

Claims 28 and 31 were rejected under 35 USC 102(b) as being anticipated by Tuggle et al. (US 5,614,364).

Applicants have canceled claims 28 and 31, thus alleviating this rejection.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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AMENDMENT — VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

On page 5, the paragraph at lines 1 and 2 has been replaced with the following:

Figure 2 represents a comparison of the DNA sequence between the human (SEQ ID NO: 2) and the porcine (SEQ ID NO: [3]1) MC4R gene.

On page 5, the paragraph at lines 3 and 4 has been replaced with the following:

Figure 3 represents a comparison of the amino acid sequence between the human (SEQ ID NO: [4]3) and the porcine (SEQ ID NO: [5]4) MC4R gene.

On page 5, the paragraph at lines 6-8 has been replaced with the following:

Figure 5 depicts an alignment of partial nucleotide and amino acid sequences of two different porcine MC4R alleles. As can be seen, the partial nucleotide sequence of allele 1 (SEQ ID NO: 27) differs from that of allele 2 (SEQ ID NO: 28) by virtue of a guanine→adenine substitution. This substitution results in a corresponding amino acid change (indicated by arrow) from aspartic acid (D) in the partial amino acid sequence of allele 1 (SEQ ID NO: 29) to asparagine (N) in allele 2 (SEQ ID NO: 30)[(SEQ ID NO: 12) of the porcine MC4R gene. The amino acid translation shows an amino acid substitution at codon 298].

On page 5, the paragraph at lines 11-18 has been replaced with the following:

Figure 7 depicts multiple-alignments of the putative seventh transmembrane domain of porcine MC4R with other MCRs and GPCRs. The "*" represents the predicted sequence positions for porcine MC4R ("pMC4R") (SEQ ID NO: 11). The other amino acid sequences ("hMC4R"; "rMC4R"; "sheep MC5R"; "bovine MC5R"; "bovine MC2R"; "hMC3R"; "mMC3R"; "hMC2R"; "hMC1R"; "bEDG-2R"; "hEDG-4R"; "human cannab"; "hH2AB"; "rSSR2"; "hGAL-R") were obtained from the GenBank database (respectively, accession

numbers P32245 (SEQ ID NO:12), P70596 (SEQ ID NO:13), P41983 (SEQ ID NO:14), P56451 (SEQ ID NO:15), P34974 (SEQ ID NO:16), P41968 (SEQ ID NO:17), P33033 (SEQ ID NO:18), Q01718 (SEQ ID NO:19), Q01726 (SEQ ID NO:20), Q28031 (SEQ ID NO:21), AF011466 (SEQ ID NO:22), P21554 (SEQ ID NO:23), P18089 (SEQ ID NO:24), P30680 (SEQ ID NO:25), P47211 (SEQ ID NO:26)). The missense variant in porcine MC4R substituted amino acid N for D in the position marked with an arrow. The Asp (D) residue is highly conserved among MCRs, and the Asn (N) residue is well conserved in most other GPCRs.

On page 6, the paragraph at lines 23-29 has been replaced with the following:

Another embodiment of the invention provides a kit for assaying the presence in a MC4R gene sequence of a genetic marker. The marker being indicative of inheritable traits of fat content, growth rate, and/or feed consumption. The kit in a preferred embodiment also includes novel PCR primers comprising 4-30 contiguous bases on either side of the polymorphism to provide an amplification system allowing for detection of the *Taq I* polymorphism by PCR and *Taq I* digestion of PCR products. The preferred primers are SEQ ID NO: [8]7 and SEQ ID NO: [9]8.

On page 7, the paragraph at lines 21-30 has been replaced with the following:

From sequence data, it was observed that in allele 2 the guanine is substituted with an adenine at position 678 of the PCR product or at position 298 amino acid of the MC4R protein changing the aspartic acid codon (GAU) into an asparagine codon (AAU). The PCR test for the polymorphism used a forward primer of 5'-TGG CAA TAG CCA AGA ACA AG-3' (SEQ. ID NO: [6]5) and a reverse primer of 5'-CAG GGG ATA GCA ACA GAT GA-3' (SEQ. ID NO: [7]6). Pig specific primers used were a forward primer of 5'-TTA AGT GGA GGA AGA AGG-3' (SEQ. ID NO: [8]7) and a reverse primer of 5'-CAT TAT GAC AGT TAA GCG G-3' (SEQ ID NO: [9]8). The resulting amplified product of about 750 bp, when digested with *Taq I*, results in allelic fragments of 466, 225, and 76 bp (allele 1) or 542 and 225 bp (allele 2).

On page 8, the paragraph at lines 8-25 has been replaced with the following:

Other possible techniques include non-gel systems such as [TaqMan]TAQMAN™ (Perkin Elmer). In this system, oligonucleotide PCR primers are designed that flank the mutation in question and allow PCR amplification of the region. A third oligonucleotide probe is then designed to hybridize to the region containing the base subject to change between different alleles of the gene. This probe is labeled with fluorescent dyes at both the 5' and 3' ends. These dyes are chosen such that while in this proximity to each other the fluorescence of one of them is quenched by the other and cannot be detected. Extension by *Taq* DNA polymerase from the PCR primer positioned 5' on the template relative to the probe leads to the cleavage of the dye attached to the 5' end of the annealed probe through the 5' nuclease activity of the *Taq* DNA polymerase. This removes the quenching effect allowing detection of the fluorescence from the dye at the 3' end of the probe. The discrimination between different DNA sequences arises through the fact that if the hybridization of the probe to the template molecule is not complete, i.e., there is a mismatch of some form, the cleavage of the dye does not take place. Thus, only if the nucleotide sequence of the oligonucleotide probe is completely complementary to the template molecule to which it is bound will quenching be removed. A reaction mix can contain two different probe sequences each designed against different alleles that might be present, thus, allowing the detection of both alleles in on reaction.

On page 10, the paragraph at lines 5 and 6 has been replaced with the following:

MC4R1: 5' TGG CAA TAG CCA AGA ACA AG 3' (SEQ ID NO: [6]5)

MC4R4: 5' CAG GGG ATA GCA ACA GAT GA 3' (SEQ ID NO: [7]6)

On page 11, the paragraph at lines 27 and 28 has been replaced with the following:

Figures 2 and 3 illustrate the differences between the DNA and amino acid sequences of the human and porcine MC4R gene (SEQ ID NOS: [2]1-[5]4).

On page 12, the paragraph at lines 20 and 21 has been replaced with the following:

Forward primer: 5'-TTA AGT GGA GGA AGA AGG-3' (SEQ ID NO: [8]7)

Reverse primer: 5'-CAT TAT GAC AGT TAA GCG G-3' (SEQ ID NO: [9]8)

In the Claims

Please cancel claims 12, 28 and 31.

Please the following claims:

1. (Thrice Amended)

A method for identifying an animal which possesses a genotype associated with [variation in] one or more favorable metabolic traits selected from fat content, growth rate, and feed consumption, the method comprising:

obtaining a nucleic acid sample from an animal; and

[assaying] detecting [for the presence of] a polymorphism at position 678 of SEQ ID NO: 1 [in the MC4R gene] wherein said polymorphism is associated with [variation in] one or more of the metabolic traits of fat content, growth rate, and feed consumption.

2. (Thrice Amended)

The method of claim 1 wherein the polymorphism is [identified] detected at position 678 of a PCR sequence [of the MC4R gene in pigs and other animals] using a forward primer and a reverse primer.

4. (Thrice Amended)

The method of claim 2 wherein the polymorphism detected at base 678 is a guanine [at base 678 in a PCR sequence of the MC4R gene] the polymorphism being associated with [variation in] fat content.

5. (Thrice Amended)

The method of claim 2 wherein [a marker] the polymorphism for lower feed intake, than animals without the marker, is [identified detected by] an adenine at base 678 [of a PCR sequence of the MC4R gene].

6. (Thrice Amended)

The method of claim 2 wherein [a marker] the polymorphism for faster rate of gain, than animals without the [marker] polymorphism, is [identified by] an adenine at base 678 of a PCR sequence [of the MC4R gene].

20. (Thrice Amended)

A method of identifying an animal which possess a [desired] genotype associated with [variation in] one or more metabolic traits selected from fat content, growth rate, and feed consumption, the method comprising:

obtaining a nucleic acid sample from an animal;

amplifying nucleic acid of said sample with primers SEQ ID NO: 5 and SEQ ID NO: 6.

sequencing the amplified product to reveal a nucleotide substitution within a *Taq I* restriction enzyme recognition site;

digesting the amplified product with *Taq I* to obtain fragments;

separating the fragments obtained from the digestion, and

generating a MC4R gene fragment having one *Taq I* restriction site with primers SEQ ID NO: 9 and SEQ ID NO: 10; and

identifying the presence or absence of a *Taq I* site

wherein the presence of a *Taq I* restriction [site] pattern in said genotype identifies the presence of a polymorphic site in the MC4R gene[associated with variation in one or more of the metabolic traits in the animal].

22. (Amended)

The method of claim 20 wherein the [site] restriction pattern generated is identifiable by fragments of 466, 225, and 76 bp [when a guanine is present at base 678 of the amplified product and fragments of 542 and 225 bp when an adenine is present when a restriction enzyme which cuts at the same recognition site as *Taq I* is used] which is indicative of an animal having lower backfat, lower daily rate of gain and lower feed intake than an animal without the polymorphism.

29. (Twice Amended)

A method for an indirect selection of a polymorphism in a MC4R gene associated with variation in one or more metabolic traits selected from fat content, growth rate, and feed consumption comprising:

[selecting specific alleles of an alternative DNA marker associated with the MC4R gene, wherein the MC4R gene is associated with a particular metabolic trait;

making an indirect selection of the polymorphism; and]

establishing a linkage between the specific alleles of [the] an alternative DNA maker and alleles of the DNA marker known to be associated with [the] a metabolic trait selected from the group consisting of fat content, growth rate, and feed consumption and

selecting for the polymorphism with the alternative DNA marker.

30. (Amended)

The method of claim 29 wherein the linked marker known to be associated with the metabolic trait is selected from the group consisting of S0331, BHT0433, and S0313.

Please add new claims 34-36:

34. (New)

The method of claim 2 wherein the forward primer is SEQ ID NO: 5 and the reverse primer is SEQ ID NO: 6.

35. (New)

The method of claim 2 wherein the forward primer is SEQ ID NO: 7 the reverse primer is SEQ ID NO: 8.

36. (New)

The method of claim 2 wherein the forward primer is SEQ ID NO: 9 and the reverse primer is SEQ ID NO: 10.

37. (New)

The method of claim 20 wherein the restriction pattern generated is identified by fragments of 542 and 225 bp which are indicative of having faster rate of gain than an animal without the polymorphism.